Title	Degradation of cis-1,4-Polyisoprene Rubbers by White Rot Fungi and Manganese Peroxidase-catalyzed Lipid Peroxidation(ABSTRACTS (PH D FOR GRADUATE SCHOOL OF AGRICULTURE))
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Degradation of *cis*-1,4-Polyisoprene Rubbers by White Rot Fungi and Manganese Peroxidase-catalyzed Lipid Peroxidation

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Natural rubber, exclusive *cis*-isoprene units linked to each other by 1,4-addition, is produced by more than 2500 different species of plants and some fungi. Natural rubber has been commercially exploited for more than 100 years by cultivating and tapping the rubber tree, *Hevea brasiliensis*. As an alternative to biological production, synthetic *cis*-1,4-polyisoprene has also been obtained since polymerization of isoprene by the Ziegler catalyst was achieved. These raw rubber materials are converted to rubber products by the process of vulcanization that leads to cross-links between rubber chains [1]. Sulfur vulcanization creates a strong chemical network that gives recalcitrance toward physical, physicochemical, and microbial destruction and superior physical properties of elasticity, whereas recycling of spent rubber products becomes problematic due to the irreversible process [2].

White rot basidiomycetes have been the focus of research with respect to the biodegradation of lignin in wood and the bioremediation of environmental hazards. White rot basidiomycetes and ligninolytic systems have also been reported to degrade natural and synthetic polymers. However, to our knowledge, the degradation of rubber products by basidiomycetes has not been reported. In this study, the degradation of vulcanized natural rubber sheets by basidiomycetes was investigated, and it was demonstrated that a white rot basidiomycete, *Ceriporiopsis subvermispora*, degraded vulcanized natural rubber sheets in wood cultures [3]. The fungus decreased total sulfur content of the rubber by 54% in 200 days, accompanied by the cleavage of sulfide bonds between the polyisoprene chains. X-Ray photoelectron spectroscopy (XPS) demonstrated that *C. subvermispora* reduced the frequency of S–C bonds by 69% with the concomitant formation of S–O bonds during the culture. Dipolar decoupling/magic angle spinning (DD/MAS) solid state ^{13}C NMR revealed that the fungus preferentially decomposed monosulfide bonds linked to a *cis*- and *trans*-1,4-isoprene backbone. When the rubber sheets were exposed to a culture of a white rot fungus, *Dichomitus squalens*, for 200 days, a 15% decrease in the total sulfur content and the formation of S–O bonds in the rubber was observed. However, a decrease in S–C bonds and an increase in the volume swelling ratio in toluene were not observed. These results indicate that *D. squalens* did not cleave S–C bonds but removed unbound sulfur or oxidized sulfide to sulfoxide. The oxidative cleavage of sulfide bonds by *C. subvermispora* demonstrates that ligninolytic basidiomycetes are microbes with the potential to devulcanize rubber products.

Lipid peroxidation (LPO) is a ligninolytic system proposed for selective white rot. The fungus, C. *subvermispora* produces unsaturated fatty acids (UFSAs) and cause extracellular LPO of the USFAs catalyzed by ligninolytic enzymes [4, 5]. In the present study, the LPO of USFAs catalyzed by oxidative enzymes and transition metals were applied to the degradation of vulcanized and nonvulcanized synthetic polyisoprene. It was demonstrated that nonvulcanized and vulcanized polyisoprene rubber materials were degraded by controlling the free radical chain reactions of lipids using oxidative enzymes, manganese peroxidase (MnP), laccase (Lac), and horseradish peroxidase (HRP) [5]. Nonvulcanized synthetic polyisoprene was degraded by the free radicals from a USFA, linoleic acid (LA) produced by MnP, HRP, and a combination of Lac/1-hydroxybenzotriazole. The degradation of nonvulcanized polyisoprene was also observed in the LPO of LA initiated by the Fenton reaction (FR) and Mn(III), an oxidation product produced by MnP. While lipoxygenase (LOX) can directly oxidize LA as a substrate, LOX caused no apparent degradation due to the differences in radical chain reactions in the LPO. Vulcanized polyisoprene rubber sheets were degraded by the LPO of LA initiated by HRP, MnP, Mn(III), and FR. Pyrolysis GC–MS analysis demonstrated that the LPO liberated isoprenoid fragments extractable with chloroform. The mechanism for the decomposition of nonvulcanized polyisoprene should be explained by hydrogen abstraction from a β-position of double bonds in isoprene chains by free radicals from lipids and the β-oxidation of the alkoxyl radical. Concerning the decomposition of vulcanized polyisoprene rubber sheets, scission of sulfide linkages in the sheets by the free radicals may be also involved in the decomposition, together with breakdown of isoprene chains.

In addition to the actinomycetes, bacteria, and fungi imperfecti previously reported as rubber-degrading microorganisms, this is the first report of basidiomycetes capable of degrading

vulcanized rubber. A white rot basidiomycete, *Ceriporiopsis subvermispora*, oxidatively cleaved sulfide bonds in vulcanized polyisoprene rubber. It was also demonstrated that *cis*-1,4-polyisoprene and vulcanized rubber products were degraded by the oxidation of unsaturated fatty acids with oxidative enzymes and transition metals. Control of free radical reactions of LPO by enzymes and transition metals will allow us to develop novel techniques for safe disposal and recycling of vulcanized rubber wastes.

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